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Systematic studies in the genus *Mohria* (Pteridophyta: Anemiaceae). II. Comparative vestiture morphology and phylogeny

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Clavate and naviculate trichomes are common to *Mohria* and *Anemia* and support their affinity and inclusion in the Anemiaceae. Hair morphology in the genera, however, differs somewhat and may be ascribed to their long and diverse evolutionary histories. Scales are found in *Mohria* only and are commonly used as a means of separating the two genera. Phylogenetic studies into *Mohria* trichomes showed hairs with straight transverse walls as plesiomorphic while sinuate-walled hairs are considered to be derived from them. Ossiform-celled hairs are interpreted as apomorphic. Scales are hypothesized as being derived from sinuate-walled and ossiform-celled hairs.

Knuppelvormige en bootvormige trigome kom in *Mohria* en *Anemia* voor en steun hul verwantskap en insluiting in die Anemiaceae. Haarmorfologie in die genera verskil egter tot 'n mate en kan toegeskryf word aan hul lang en diverse evolusionêre geskiedenisse. Skubbe word alleenlik in *Mohria* aangetref en word gebruik om die genera te onderskei. Filogenetiese studies in die trigome van *Mohria* toon hare met reguit dwarswande as plesiomorfies terwyl hare met gekartelde dwarswande daarvan afgelei is. Ossiforme hare word as apomorfies beskou. Skubbe ontstaan uit hare met gekartelde dwarswande en uit ossiforme hare.

Keywords: *Anemia*, Anemiaceae, *Mohria*, phylogeny, trichomes.

Introduction

Actinostachys Wall., *Anemia* Swartz, *Lygodium* Swartz, *Mohria* Swartz and *Schizaea* J. Smith are all extant members of the schizaeoid ferns. They are generally placed in a single family, the Schizaeaceae, because of their exindusiate, monosporangiate sori and characteristic apical ring of annulus cells. The monophyly of this assemblage has been questioned by Reed (1947), Bierhorst (1971), Löve *et al.* (1977) and De la Sota and Morbelli (1987). Reed (1947) gave familial status to *Anemia* (Anemiaceae) and *Mohria* (Mohriaceae) but this classification did not gain much support and has since been rejected. A system whereby both genera are included in the Anemiaceae has been accepted by Bierhorst (1971), Löve *et al.* (1977) and De la Sota and Morelli (1987).

Mohria and *Anemia* are separated by their diverse distribution of fertile pinnae on the lamina. In *Mohria* the fertile pinnae occur distally whereas in *Anemia* they largely occur proximally. Scales in *Mohria* and their absence from *Anemia*, where only hairs occur, also separate the genera.

In preparing a revision of the genus (Roux 1990) I found trichomes to be of taxonomic importance. The aim of this paper is to conduct comparative studies into the trichome types of *Mohria* and *Anemia* and to provide a hypothesis of their phylogeny in *Mohria*.

Material and methods

Trichomes were obtained from live material and selected herbarium sheets, cleaned and cleared with household bleach, rinsed in clean water and mounted in glycerine. Camera-lucida drawings were done using a Leitz 'Laborlux K' microscope and drawing tube. For scanning electron microscopy fresh material was fixed in FAA and dehydrated in a graded ethanol series. After critical point drying the material was sputter-coated with Au/Pd and viewed with a Cambridge S200 microscope at 20 kV.

Trichomes of all the *Mohria* species, *i.e. M. caffrorum* (L.) Desv., *M. lepigera* (Baker) Baker, *M. marginalis* (Sav.) J.P. Roux, *M. nudiuscula* J.P. Roux, *M. rigida* J.P. Roux, *M. saxatilis* J.P. Roux and *M. vestita* Baker, have been studied. For comparative purposes the trichomes of *A. dregeana* Kunze and *A. simii* Tardieu-Blot have also been studied.

Discussion

Indumentum in *Mohria* consists of four trichome types, namely unicellular clavate-type trichomes, unicellular naviculate-type trichomes, hairs and scales (Roux *et al.* 1992).

Unicellular clavate-type trichomes (Figure 1R)

Clavate trichomes are thin-walled structures and are found in all the species. These presumably glandular obovoid or clavate structures are borne on small randomly dispersed epidermal cells with anticlinal walls which are less contorted than the surrounding epidermal cells. They occur more commonly on the abaxial surface of the lamina and are also more frequent on juvenile fronds. In *M. saxatilis*, however, they tend to be restricted to the veins. In some plants of this species clavate trichomes were not observed adaxially on the lamina and in some instances they may be lacking entirely. Clavate trichomes are basally attached. Their length ranges from 30 to 76 μm (Table 1).

Clavate trichomes are of no apparent taxonomic value. The relatively large trichomes of *M. caffrorum* and *M. saxatilis*, however, suggest a phylogenetic relationship.

Clavate trichomes have also been reported in *Anemia* by Mickel (1962), who suggested their length to be correlated with that of the stomata and hence to reflect the ploidal level of the plant. This could not be confirmed for *Mohria*. In *Anemia* their length ranges between 32 and 70 μm.

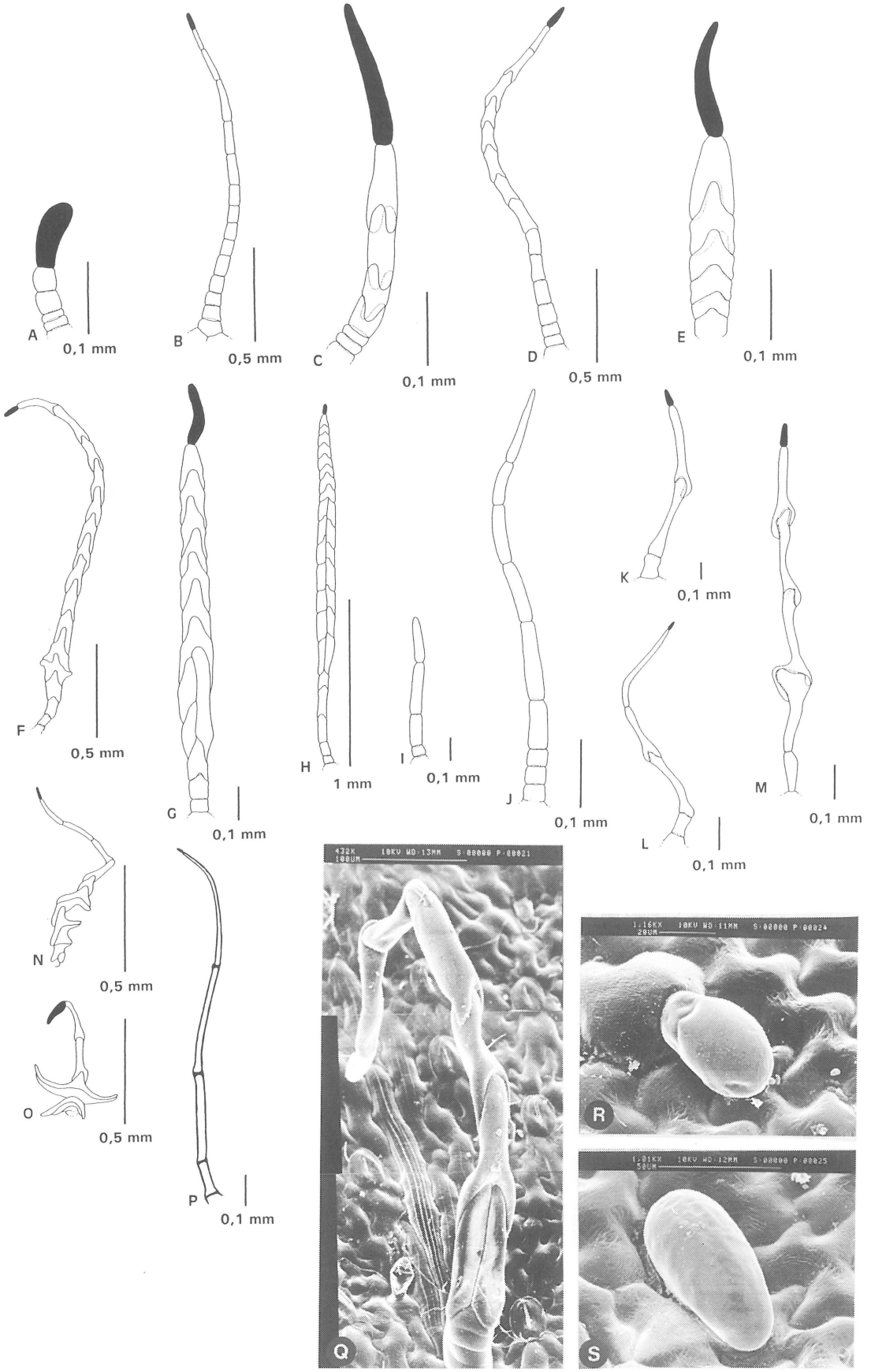

Figure 1 Trichome types in *Mohria* and *Anemia*. Glandular straight-walled hairs in: **A**. *M. marginalis* (*Roux 907*, NBG); **B**. *M. marginalis* (*Demarne s.n.*, NBG). Proximally straight-walled, distally sinuate-walled hairs in: **C**. *M. marginalis* (*Roux 907*, NBG); **D**. *M. marginalis* (*Demarne s.n.*, NBG). Sinuate-walled hairs in: **E**. *M. marginalis* (*Roux 907*, NBG); **F**. *M. marginalis* (*Demarne s.n.*, NBG). Hair-like scales in: **G** & **H**. *M. marginalis* (*Roux 907*, NBG). Eglandular hair in: **I** & **J**. *M. marginalis* (*Roux 907*, NBG). Ossiform-celled hairs in: **K** & **M**. *M. vestita* (*Steiner s.n.*, NBG); **L**. *M. vestita* (*Roux 1900*, NBG). Scale-like hairs in: **N** & **Q**. *M. nudiuscula* (*Schelpe 4010*, BOL); **O**. *M. nudiuscula* (*Esterhuysen 12894*, BOL). Glandular lamina hair in: **P**. *A. dregeana* (*Roux 551*, NBG). Clavate trichome in: **R**. *M. marginalis* (*Roux 907*, NBG). Naviculate trichome in: **S**. *M. marginalis* (*Roux 907*, NBG).

Table 1 Mean length of unicellular clavate-type trichomes

Species	S.D.	$\bar{x}$ (μm)	*n*	Range (μm)
M. caffrorum	3.92	59.15	50	55.0 – 67.5
M. lepigera	3.65	42.52	50	30.5 – 48.8
M. marginalis	3.32	48.75	50	37.5 – 57.5
M. nudiuscula	4.70	50.50	50	37.5 – 62.5
M. rigida	4.85	45.76	77	35.0 – 61.1
M. saxatilis	3.96	68.11	61	61.1 – 76.4
M. vestita	4.65	51.31	100	30.0 – 63.4

Table 2 Mean length of unicellular naviculate-type trichomes

Species	S.D.	$\bar{x}$ (μm)	*n*	Range (μm)
M. caffrorum	19.83	179.80	50	147.5 – 222.5
M. lepigera	20.26	113.44	50	50.0 – 147.5
M. marginalis	16.65	104.30	50	57.0 – 145.8
M. nudiuscula	14.40	156.40	50	120.8 – 190.2
M. rigida	28.26	112.86	84	77.5 – 164.97
M. saxatilis	15.90	139.05	54	97.7 – 177.19
M. vestita	20.50	138.32	100	85.0 – 196.54

Unicellular naviculate-type trichomes (Figure 1S)

Naviculate trichomes are found in all the species. They are thin-walled, ellipsoid, narrowly ovoid or oblong structures and are presumably glandular. Their contents appear crystalline when dry and on older fronds are pale yellowish in colour. Naviculate trichomes are basally or sub-basally attached to epidermal cells which are much smaller than the adjacent cells and also have less contorted walls.

On the lamina they are more commonly found abaxially. They are also associated with the teeth or lobe apices of the fertile pinnae where they occur in large numbers. The possible purpose of the association with sporangia is unknown as they would provide little physical protection. They may, however, serve as a chemical defence. In juvenile fronds these trichomes are common on both lamina surfaces but appear to be shed as ageing sets in. Naviculate trichomes also occur on the stipe and rachis where the density is variable.

The length of the trichomes varies considerably. The shortest trichomes were observed in *M. lepigera* (50 μm) and the longest in *M. caffrorum* (222 μm) (Table 2). Their width ranges from 12.5 to 25 μm.

Ontogenetic studies show naviculate trichomes to develop from a slightly smaller, wedge-shaped superficial initial through periclinal division. Initially the attachment appears basal, but this position is mostly replaced by a lateral attachment in the mature state. Naviculate trichomes appear to be of little taxonomic value.

Naviculate trichomes also occur in *Anemia* (Mickel 1962) where their length ranges between 78 and 172 μm.

Hairs

Hairs occur in all the species and are found on the stipe, rachis and lamina surfaces. They develop from a single superficial initial by periclinal division. Initially the basal cell of the hair is of the same size as those surrounding it, but at maturity they often are larger. The basal cells of hairs along the veins are not significantly enlarged. Superficially these cells are characterized by less contorted anticlinal walls than the surrounding epidermal cells. The mature basal cell is furthermore conspicuously elevated.

Hairs occurring in the genus can be divided into two groups, namely, gland-tipped hairs, and eglandular hairs.

Gland-tipped hairs are the most common type in *Mohria*. In these hairs the apical cell is similar in structure and contents to naviculate trichomes. Gland-tipped hairs may again be divided into groups with straight transverse walls (Figures 1A & 1B) and more commonly those with sinuate transverse walls (Figures 1E & 1F). Intermediates between the two types are not uncommon (Figures 1C & 1D).

Hairs with straight transverse walls are more commonly found in *M. lepigera, M. marginalis* and more rarely in *M. saxatilis*. The basal cells of these structures are shorter (25 – 30 μm) than those higher up (55 – 77 μm). Hairs in *M. marginalis* may be 328 – 620 μm long, whereas those on the adaxial lamina surface of *M. lepigera* are 237 – 320 μm long.

A variation of the sinuate-walled type, here termed ossiform-celled, occurs (Figures 1K – 1M). The joints in ossiform-celled hairs are usually asymmetric, much enlarged and are often oblique (Figure 1M). Ossiform-celled hairs, commonly found in *M. nudiuscula, M. rigida* and *M. vestita*, are most frequent adaxially along the secondary rachis, costa and veins. In some events the proximal cells are remarkably shorter (10 – 50 μm) than the distal ones (92 – 185 μm long). Ossiform-celled hairs may reach a length of up to 2.7 mm.

Eglandular hairs are known in some collections of *M. marginalis* only and always have straight transverse walls (Figures 1I & 1J). They largely occur on the adaxial lamina surfaces. Mickel (1962) suggested glandular hairs in *Anemia* to be the primitive state and eglandular hairs to be derived. This is in conflict with ontogenetic evidence and eglandular hairs are hence considered plesiomorphic by me. As eglandular hairs are present in certain collections in *M. marginalis* only, they do not contribute to or resolve the phylogeny of the *Mohria* species.

Hairs in *Anemia* are unicellular or multicellular (Mickel 1962). Multicellular hairs consist of a short basal cell and three to six elongated cells (Figure 1P). The hairs in *A. colimensis* Mickel are unique in that they are unicellular, stiff and have thickened walls. *Anemia* species I have investigated had hairs with straight transverse walls which showed secondary thickening and were often septate. Apart from species in the subgenus *Anemiorrhiza*, hairs in *Anemia* are always gland-tipped.

Scales

Scales are common epidermal appendages in the leptosporangiate ferns and have obviously arisen more than once through parallel evolution. In the schizaeoid ferns simple uniseriate hairs are prevalent but in *Mohria* scales are interspersed with hairs.

Scales are one cell layer thick and are found on the rhizome, stipe, rachis and lamina. They are always gland-tipped. On the lamina their distribution varies. In most taxa

scales are restricted to the abaxial surface of the lamina, while in *M. nudiuscula*, *M. rigida* and *M. vestita* small scales often occur adaxially along the rachis and secondary rachis. Scale density varies considerably within a species.

Scale outline is remarkably variable and ranges from filiform to narrowly linear, cordate, broadly ovate, hastate, or with the basal part circular or transversely ovate which abruptly terminates into a long and narrow filiform apex.

Scale attachment may be sessile, but in cordate or cordate-imbricate scales they are attached by a small group of cells, or a number of convexly arranged cells. Rhizome scales are always sessile while those along the stipe are either sessile or more commonly cordate. Lamina scales are usually attached by one to three cells only but in *M. saxatilis* they are often short-stalked and attached to the surface by several cells. Scales occurring adaxially on the lamina are usually attached by a single cell.

Rhizome scales are generally longer than the lamina scales and are usually narrow and entire. They are darker in colour and more lignified than those on other parts of the plant.

Stipe and rachis scales differ from rhizome scales in size and margin sculpture. In some species they consist of what appears to be more than one type. In *M. nudiuscula* and *M. rigida*, scales on the adaxial surface of the rachis are often smaller and differ in form from those on the abaxial surface.

In *M. marginalis* and *M. lepigera*, scale margins are generally entire or shallowly and irregularly crenulate. In other taxa the margins are more varied. In most cases, however, they are distally entire and proximally with short and/or long cellular outgrowths.

The cell structure of the scales shows two patterns. The first type is illustrated by some plants of *M. caffrorum*, especially from the Gifberg in the north-western Cape, *M. marginalis* and *M. lepigera*. Here the walls are deeply sinuate, but proximally the cells become like the second type, *i.e.* straight-walled. Although they are called straight-walled the cells are often irregularly shaped. In most cases the cells are arranged longitudinally but often the orientation of the marginal cells changes and they become transversely arranged.

Scale ontogeny shows the primordia to be hair-like and made up of a uniserial row of straight-walled cells (Figure 2A). Divergence, however, soon takes place as the structures become multicellular and flattened in the upper parts while the cells of the basal part remain straight-walled. Initially the scales are narrowly linear but as a result of anticlinal divisions they later consist of two or more rows of cells (Figure 2B). At this stage the scale orientation is perpendicular to the lamina surface.

With further development the scales become broader (Figures 2C – 2H). Initially the base is cuneate (Figure 2D) but later it becomes cordate (Figure 2G) or imbricate-cordate (Figure 2F). The scales now lie parallel to the lamina surface with the apices facing distally. Developing scales are colourless but they soon become lignified and turn brown.

The margins are initially always entire but as a result of the meristematic proximal part the margins in mature scales are often fimbriated. Fimbriated margins appear to be the result of transverse divisions as the cell orientation along the margins is largely in that direction.

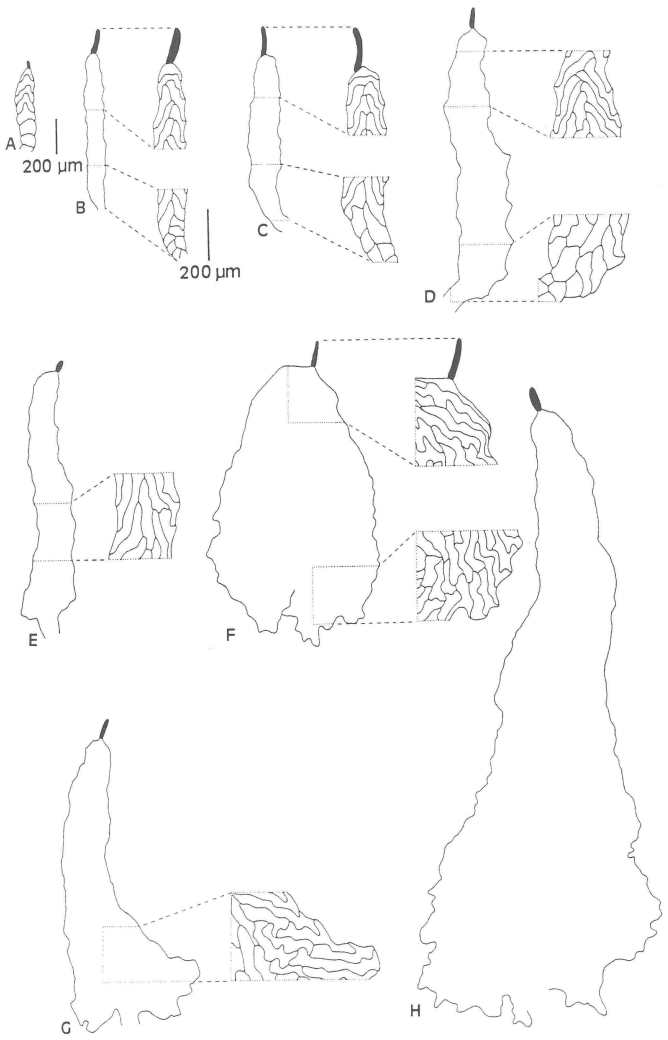

Figure 2 Lamina scale ontogeny in *Mohria caffrorum*. **A – H**. Scale outlines and sections showing the cellular structure (*Duncan s.n.*, NBG).

Conclusion

Character evolution was polarized by ontogenetic evidence (Wiley 1981; Radford 1986) and outgroup comparison (Watrous & Wheeler 1981; Wiley 1981).

Clavate and naviculate trichomes found in *Mohria* and *Anemia* are similar in structure and contents and are obviously homologous. They are seen here as synplesiomorphic characters.

Ontogenetic studies showed hairs to be uniseriate, gland-tipped and with straight transverse walls (Figure 1A). In some species hairs with straight transverse walls give rise to glandular sinuate-celled hairs. Here the proximal cells are always short and straight-walled while distally they become longer and sinuously jointed (Figure 1D). Studies also showed ossiform-celled hairs to derive from the sinuate-walled type. On the principle of outgroup comparison and ontogeny, glandular straight-walled hairs are here considered plesiomorphic while the sinuate- and ossiform-celled types are considered derived.

The presence of scales in *Mohria* separates it from *Anemia*. Schoute (1938), Nayar (1962), Wagner (1964) and Mickel (1981) considered scales merely as flat multicellular hairs. As a result of their presence, however, Bower (1923) and Mickel (1962) considered *Mohria* to be the most

advanced member of the family.

In *M. marginalis*, trichomes which are morphologically strikingly different occur. Here a continuous transition between hairs and scales exists. Filiform scales which are uniseriate at the base but sinuately-walled, multiseriate, and flattened higher up are frequently found (Figures 1G & 1H). These structures are likely to occur in a state of arrest and are considered as neotenic forms. They are replaced by broader scales with multicellular attachments. These stages may be considered as representative of the scale phylogeny in *Mohria*.

However, in *M. nudiuscula, M. rigida* and *M. vestita* ossiform-celled hairs on the adaxial surface of the lamina are often replaced by small scales (Figures 1M – 1O). These scales are obvious elaborations of ossiform-celled hairs as the distal cells become longer while the proximal cells generally become transversely elongated. They are here considered derived. Thus, scales in *Mohria* are derived in two different ways.

Vestiture in the Anemiaceae is also diverse. Based on out-group comparison and the controversial assumption of correlation (Radford 1986), Mickel (1962) suggested hirsute laminae as plesiomorphic and glabrous laminae as apomorphic. Laminae which are adaxially hirsute are only known in *M. lepigera* and *M. marginalis*, both of which are characterized by short-celled hairs with straight transverse walls. Out-group comparison in *Mohria* supports the hypothesis whereby adaxially hirsute laminae set with straight-walled hairs represent the ancestral state and the adaxially glabrous or near-glabrous laminae set with ossiform-celled hairs and small scales are derived.

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